A New Approach to an Old Problem: Devising Formulas To Enhance Growth

wo researchers at the U.S. Horticultural Research Laboratory in Fort Pierce, Florida, are attracting international attention in diverse scientific fields for their approach to a common problem in science: how to predict every possible variable in an experiment where you simultaneously manipulate numerous components.

Researchers who use culture media to grow and study cells, tissues, or any biological entity typically try to optimize the media by varying one ingredient at a time, while holding all the other ingredients constant. The problem is that changing the amount of any single ingredient in a mixture changes not only the amount of that ingredient, but also its proportion to every other ingredient in the mix. The one-ingredient-at-a-time—or one-factor-at-a-time—approach fails to take into account the effects of proportions. In addition, this can be an expensive and time-consuming process, with no significant improvement in culture growth.

But geneticist Randall P. Niedz and ecologist Terence J. Evens have developed a new approach that can help researchers reformulate any type of mixture, whether it is a culture medium, fertilizer, potting soil, insect diet, or animal feed.

Niedz and Evens encountered the problem a few years ago while each was studying mineral nutrients. Niedz was trying to improve the growth of citrus tissue culture, an important area for crop improvement, but one that tends to be a challenge. Evens was studying the effects of algal growth in runoff from ornamental plants in nurseries.

A typical plant/algae culture medium contains about 16 individual mineral nutrient ions, and its effects are determined by both the amount and the proportion of each ingredient in the mix. Niedz and Evens recognized that creating a nutrient solution is a special type of mixture problem and should be defined by the composition of its individual ions. They found that by examining





Citrus cell lines growing on standard media formulation (left) and on a formulation identified after a single experiment using the new ion-based approach. The cells on the new formulation are growing almost 200 percent faster. The new approach can be used to make better formulations for many things, including potting soils, animal diets, fertilizer blends, and algae nutrient solutions.



Geneticist Randall Niedz (left) and ecologist Terence Evens inspect two green algae cultures for an ongoing study of the effects of fertilizers on algal growth.

ions independent of their parent salts, they could make sense of the specific ion effects in their solutions.

The method they developed of reconfiguring a "recipe" is complex and must be done with a computer using both an algorithm they devised and experimental designs that separate out the effects of proportionality and amount. Results are teased out in ways that reveal every possible outcome and are considerably more accurate, resource efficient, and knowledge rich than results from standard one-ingredient-at-a-time approaches, the researchers say. When applied to citrus tissue and algal culture, the results were dramatic, saving time and money and enhancing cell growth. In the citrus experiments, growth of citrus tissue cultures increased nearly 200 percent. In the algal experiments, they found unusual mixtures where algae grew vigorously and at pH levels far beyond where traditional science said they should grow.

The approach developed by Niedz and Evens is being used not only by scientists in their fields, but also by entomologists, medical researchers, and even chemists around the world to gauge the effects of varying ingredients in mixtures. They ex-

plained the approach in a letter to *Nature Methods* in June 2006 and a report in *Scholarly Research Exchange* in May 2008. They have also released a free online software package, for mineral nutrient research, that has been downloaded more than 300 times by scientists in 39 countries. The software package is available at www.ars.usda.gov/services/software/download.htm?softwareid=148.—By **Dennis O'Brien,** ARS.

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